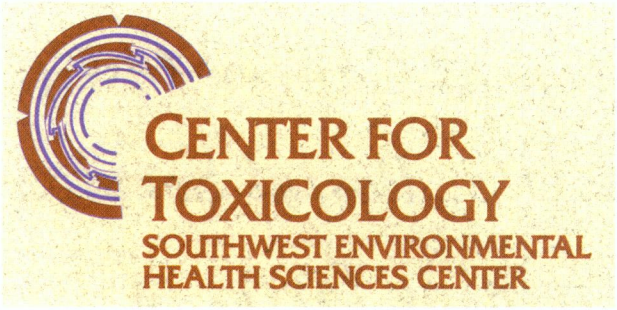
Science among the Saguaros



The Southwest Environmental Health Sciences Center (SWEHSC) thrives in the arid Southwest. Drawing from nearly 20 departments and five colleges at the University of Arizona in Tucson, the center offers investigators a forum to integrate and expand their research in the environmental health sciences.

The university's Center for Toxicology was founded in 1987. In 1994, the SWEHSC was established as a branch of the original center. According to I. Glenn Sipes, professor of pharmacology and toxicology and director of the center, the new department's name "reflected what we wanted to develop, a center of excellence in environmental health sciences that would serve the southwestern United States, as well as northern Mexico."

In addition to scientific research, the center provides outreach and education. Imaginative partnerships with elementary, middle, and high school teachers bring toxicology to classrooms, helping to encourage future scientists and scientifically literate citizens. Center scientists participate in outreach by helping community groups and other lay audiences understand toxicological issues. The center also provides courses to health care professionals to learn more about toxicology.

Through the Center for Toxicology's Hazardous Waste Basic Research Program, the SWEHSC interacts in environmental science and toxicology programs in Mexico. Their involvement includes participating in joint research projects with Mexican scientists, as well as sharing research findings with their Mexican counterparts. Specific areas of interest include chemical exposures along the border and pulmonary effects due to air pollution in Mexico City. Their involvement extends from border communities to formal courses at the university in Mexico City. Sipes says, "In Mexico, they are really impacted by environmental chemicals. What we're trying to do is to transfer the information that we have to Mexico, so they can apply it to their own particular needs and not have to 'reinvent the wheel.'"

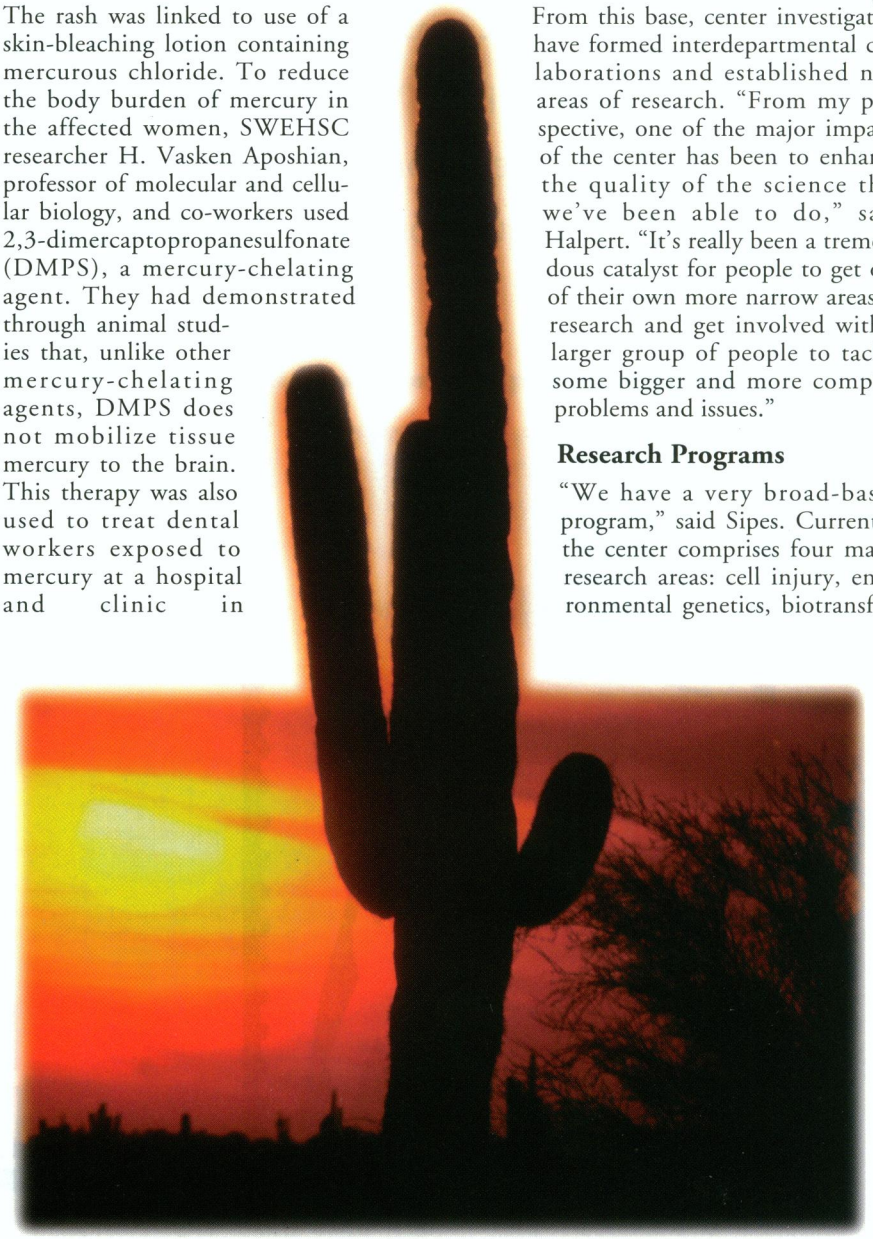
A recent collaborative project involving SWEHSC researchers was initiated by Diego Gonzalez-Ramirez, head of the Department of Pharmacology at the Instituto Mexican del Segun Social in Monterrey, Mexico. This collaboration addressed an outbreak of skin rash among Hispanic women in the southwestern United States and Mexico. The rash was linked to use of a skin-bleaching lotion containing mercurous chloride. To reduce the body burden of mercury in the affected women, SWEHSC researcher H. Vasken Aposhian, professor of molecular and cellular biology, and co-workers used 2,3-dimercaptopropanesulfonate (DMPS), a mercury-chelating agent. They had demonstrated through animal studies that, unlike other mercury-chelating agents, DMPS does not mobilize tissue mercury to the brain. This therapy was also used to treat dental workers exposed to mercury at a hospital and clinic in Monterrey. Aposhian and his colleagues suggest that the laboratory studies and demonstrated clinical effectiveness of DMPS support its use as a treatment for mercury poisoning.

The center also supports researchers and students at Mexican universities. "We've developed a number of nice collaborative interactions and have met a lot of colleagues," said Sipes. "I think it's really beneficial to scientists on both sides of the border. And, it's really critical because chemicals don't recognize the boundary."

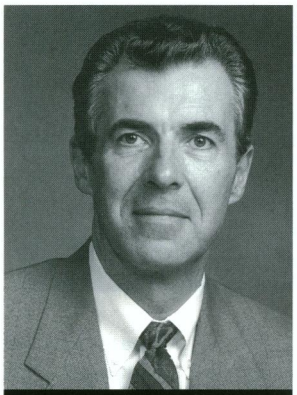
According to James Halpert, professor of pharmacology and toxicology and deputy director of the center, the University of Arizona has a strong tradition in mechanistic toxicology. From this base, center investigators have formed interdepartmental collaborations and established new areas of research. "From my perspective, one of the major impacts of the center has been to enhance the quality of the science that we've been able to do," said Halpert. "It's really been a tremendous catalyst for people to get out of their own more narrow areas of research and get involved with a larger group of people to tackle some bigger and more complex problems and issues."

Research Programs

"We have a very broad-based program," said Sipes. Currently, the center comprises four major research areas: cell injury, environmental genetics, biotransfor-



mation, and metal toxicity. These programs were formed based on the research interests of the initial center members. As the center grows, the exact makeup of the research is expected to evolve to include other programs. Cross-program collaborations are common, and many investigators are involved in more than one research program.

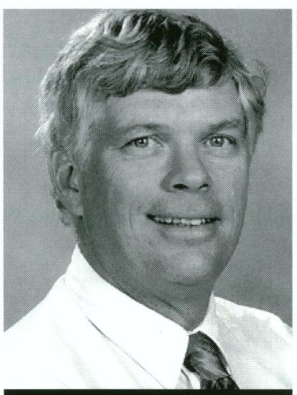

SWEHSC

I. Glenn Sipes

The biotransformation research program has been described as the cornerstone of the SWEHSC because many of the center's members have a background in biotransformation. Halpert's research is one example. Halpert, who is also the biotransformation program leader, studies the cytochrome P450 system. The P450s are a major family of enzymes in the liver and other organs that detoxify a multitude of foreign compounds. Cytochrome P450s are also responsible for bioactivating certain other chemicals or inducing toxicity by metabolism.

"Our approach is a very basic molecular one to try to understand the specificity of these enzymes," Halpert explained. Halpert and his colleagues also consider how certain chemicals may interact with others via cytochrome P450s. For example, one particular form of the enzyme, cytochrome P450 3A4, seems to be involved in drug interactions in the liver. These interactions can have serious consequences, Halpert explains, so it's important to characterize the active site of the enzyme to be able to predict potential interactions.

Charlene McQueen, associate professor of pharmacology and toxicology and leader of the environmental genetics research program, says this program has brought together investigators interested in DNA. The two main aspects of the program are how chemicals alter DNA and how genetic variation affects individual susceptibility. McQueen's research focuses on *N*-acetyltransferase polymorphism and its role in developmental toxicity. How this enzyme functions is unclear, but there is evidence that it metabolizes aromatic amines and hydrazines, compounds found in cigarette smoke and some environmental pollutants. McQueen and her colleagues have found that the genes associated with *N*-acetyltransferase are expressed during embryonic development. Therefore, she argues, both fetal and maternal biotransformation need to be researched.


SWEHSC

A. Jay Gandolfi

"Another unique area that we work on here is to understand how chemicals affect the ovary," said Sipes. "I think this is an underexplored area." In collaboration with Sipes and other investigators, Patricia Hoyer, professor of physiology, is studying chemicals that destroy the preantral follicles in the ovaries. This is a vital health reasearch topic, Hoyer says, because destruction of these follicles leads to ovarian failure and premature menopause. Ultimately, these compounds may be triggers for ovarian cancer. "We've termed this a 'silent' form of toxicity, such that there would be no traumatic inflammatory response, and a woman would never know that this is going on," Hoyer explained. Using the industrial chemicals butadiene and its derivative 4-vinylcyclohexene, Hoyer and her colleagues have demonstrated in rats and mice that the ovarian damage occurs through accelerated apoptosis, or natural cell death. Ultimately, they hope to compile an ovotoxic index that would rank chemicals according to their potential to cause ovarian damage.


SWEHSC

Patricia Hoyer

Interactive toxicology is a vital component of several center research projects, including Sipes' interest in how vitamin A modulates hepatotoxicity via its effect on Kupffer cells in the liver. "We're primarily interested in synergistic responses and antagonistic responses, and understanding what are the cellular and molecular mechanisms of these responses," he said. "Ultimately, what this [research] is trying to do is to set up how we can better understand exposure to complex mixtures."

Interactive toxicology is also a component of research done by A. Jay Gandolfi, professor of pharmacology and anesthesiology, on metals and their toxic effects in the kidney. Gandolfi says it is important to find out how metals are presented at the site of toxicity and which system is involved in taking up metals. This same theory applies to his research on the water pollutant dichloroethylene and liver toxicity. "Understanding how a toxicant gets into a cell in the correct form is as important as knowing if the compound is toxic inside the cell," he said.

Innovative Techniques and Modeling

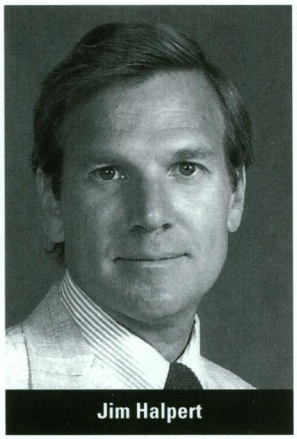
"I think one of the strengths of our cell injury program is the diversity and the sophistication of the techniques that are used," Gandolfi said, citing examples such as *in situ* microscopy and the study of single perfused nephrons.

One innovative technique used at the center is precision-cut tissue slicing. About 12 years ago, Gandolfi, along with center colleague Klaus Brendel, professor of pharmacology, developed this technique as an improvement on hand-cut slicing. Good results were possible with hand-cut slicing, but consistency was lacking. With the precision-cut slicer, investigators can cut 250-micron tissue slices whose thicknesses vary less than 10%. Incubation is the key step in this technique—incubation must be dynamic to account for gas exchange, nutritive absorption, and waste excretion, so different protocols are used depending on the tissue type. Cells generally survive 24–48 hours, but longer incubation periods of up to five days are possible.

According to Gandolfi, an advantage of this technique is that "the slices maintain a lot of the characteristics of the tissue, so they biotransform compounds almost identically to what they do *in vivo* and they maintain a lot of their functionality." Gandolfi said they've even seen cases of site-specific injury *in vitro*, which was very representative of damage

in vivo. The investigators have been able to look at tissues from a variety of species, including humans, as well as several organ types. "The beauty of it all is that you can do all your studies in these tissues and not have to make that leap of faith from animal studies to what's going to happen in humans, at least at the *in vitro* level," Gandolfi said.

Halpert and his colleagues have found molecular modeling to be an invaluable technology in their studies of cytochrome P450. Halpert explains that protein structures are often identified by X-ray diffraction, a process in which a purified protein is crystallized and bombarded with X rays. The diffraction pattern cast by the X rays allows investigators to deduce a protein's structure. However, membrane-bound proteins, such as the mammalian cytochrome P450s, are very difficult to crystallize. Bacterial P450s are soluble, though. Basing computer models on these proteins allows simulation of models of human and other mammalian P450s. "These models have proven to be amazingly useful in terms of understanding experimental results and also in making predictions of what's likely to happen with a new compound," Halpert said.


SWEHSC

Jim Halpert

The service cores and facilities at SWEHSC help investigators meet the demands on modern toxicology for cutting-edge technology. Devoting resources to purchasing and mastering the necessary technical analytical equipment would be an insupportable burden for individual investigators. By functioning in this way, "the service cores and facilities are really an integral part of the center," said Sipes, "and they probably are, along with the administrative core, what really holds the center together."


SWEHSC

Charlene McQueen

Outreach and Education

A strong outreach program features the center's involvement with schoolchildren. Currently, the SWEHSC and Rutgers University are sharing a grant to bring toxicology to students through a program called the Toxicology Risk Assessment and Pollution Network, or ToxRAP. "What we're trying to do is to provide a way for teachers to have current scientific information in their classrooms and to provide ways of making science exciting," said McQueen, the outreach program leader.

As part of another project called Integrating High School Science through Toxicology, the center brings cutting-edge research into high school classrooms. Two center researchers, Clark Lantz, associate professor of cell biology and anatomy, and Mark Witten, associate professor of pediatrics, have shared data from their research on the effects of cigarette smoke on airway development in mice with students in the program. Students have the opportunity to look at the actual data on CD-ROM, perform their own analyses, and then compare their results with the scientists' conclusions. "One reason [the outreach program] works is the fact that we have enthusiastic and eager scientists here who are willing to do these things," said McQueen.

Future Direction

Woven into the center's mission is the idea that the center will evolve over time. Sipes describes this process as fine-tuning the center to strengthen it. This continuous evolution also delivers fresh challenges to investigators. "One of the things we find as principal investigators is, after you've been working in an area for a long time, that's where your credibility is," said Halpert. "The center enables those interactions . . . that allow each of us to broaden our interests."

Julia R. Barrett